In-line Comparison of Particle Sizing by Static Light Scattering, Time-of-Transition, and Dynamic Image Analysis

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Abstract

Particle size analysis is important in both process and quality control. Different techniques are currently available. In this contribution, the characteristics of three techniques, based on Static Light Scattering (SLS), Time-of-Transition (TOT), and Dynamic Image Analysis (DIA), are compared using various aqueous dispersions. Hereby, the techniques were connected in series, so that simultaneous measurements could be performed on the same sample. The experimental results demonstrated that each of the investigated techniques has its strengths and limitations. Thus, SLS results may be largely affected by the choice of the refractive index of the dispersed particles as well as by the choice of the inversion algorithm to convert the angular spectrum to a particle size distribution. As neither TOT or DIA require information concerning the (complex) refractive index of the particles and are based on the detection of individual particles, these techniques are claimed to be very useful for measuring particles in the micrometer size range, although the measurement can be heavily affected by the particle transparency and concentration. Furthermore, all the techniques appear more suited to discerning small particles within a population of large particles than to detecting large particles within a population of small particles. Finally, TOT is much less sensitive towards submicron particles, as compared to SLS. The latter technique does not only have a broader dynamic range, which extends down to the submicron range, but also produces reliable results at higher sample concentrations as compared to TOT and DIA.

Keywords: aqueous dispersions, image analysis, laser light scattering, particle size analysis, time-of-transition

1 Introduction

The particle size distribution is an important feature of many products, ranging from powders over suspensions to emulsions, determining not only physical properties, such as flowability and floodability, but also the visual aspect and sensorial properties [1,2]. During the last few decades, static light scattering (SLS) has become the method of choice for rapid and reproducible particle sizing of dilute dispersions within the super-micron range. Although these techniques are still often denoted as small angle laser light scattering (SALLS) or laser diffraction (LD), modern devices are able to record and process the scattering pattern over a wide range of scattering angles, thus allowing the measurement of broad particle size distributions.

During recent years, alternative techniques, based on the time-of-transition (TOT) principle, as well as dynamic image analysis (DIA), have become available. The former technique is based on the extinction time of a rotating laser beam by a particle, in which a detection algorithm eliminates signals from intersections that are

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not crossing the particle in the middle and thus rejects all chord lengths that do not correspond to the true particle diameter [3]. The dynamic image analysis technique (DIA) is based on automated, software-based microscopic analysis of a large number of images [4].
In this study the features, advantages, and limitations of these three techniques have been explored using a variety of samples, differing both in particle size distribution and chemical composition. In order to eliminate sample heterogeneity as a source of variability, the techniques were coupled so that measurements could be performed during continuous recirculation of the sample through the serially connected instruments.

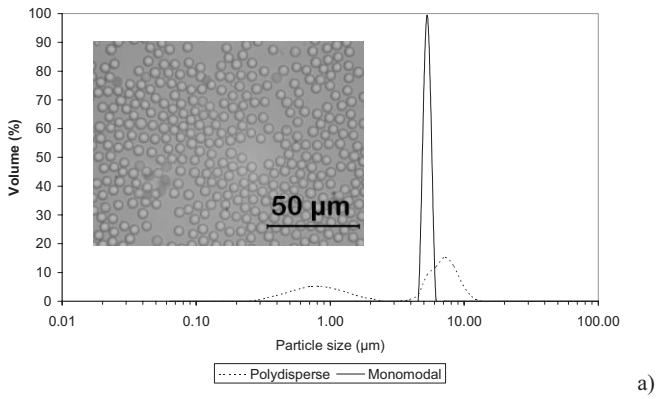
2 Materials and Methods

Monodisperse polymethylmetacrylate (PMMA) latex beads (type R-TCE6) from Agfa-Gevaert (Belgium) had a size of 5.36 ± 0.39 μm according to specifications given by the manufacturer, which was confirmed by optical microscopy. The glass beads AQ313 and AC were from Sovitec (Belgium). According to the manufacturer, AQ313 contains particles smaller than 44 μm, whereas AC contains particles whose sizes range from 149 to 250 μm; the particle density is 2475 kg/m^3. Raw milk was obtained from a local farm and was used within 24 hours to prevent microbial degradation.
Both a Mastersizer S (Malvern), equipped with a 632.8 nm red laser, 300 RF lens and MSX-17 sample dispersion unit, and a CIS-100 (Ankersmid), with GCM-104A flow through cell, were coupled in series. In the Mastersizer software the real refractive indices chosen were 1.468 for latex beads, 1.515 for glass beads, and 1.472 for milk, unless otherwise noted. The refractive index for water was set to 1.33 and the imaginary refractive index was set to zero in all cases. The polydisperse analysis model was chosen unless otherwise noted. The pumping and stirring speeds of the MSX-17 sample dispersion unit were fixed at 50 and 100 % of the maximum value, in order to avoid undesired sedimentation effects in the sample unit or connection tubes. Ten repeat measurements were performed with an acquisition time of 30 seconds (1.5*10^5 samples per measurement cycle). The dynamic image analysis (DIA) option of the CIS-100 used the WShape software. Following light correction, contrast enhancement was performed and threshold and focus levels were selected. Subsequently, 5000 particles were analyzed using the DW lens, enabling the measurement of particles within the 10–600 μm range. The Time of Transition (TOT) option used the WCIS software and A100 lens. Ten repeat measurements were performed within the size range 2–600 μm with an acquisition time of 30 seconds for the glass beads, guaranteeing that at least 10^5 particles were counted in total. For the milk particles, sample size 3 was chosen (an intermediate setting between sampling time and accuracy), and a size range of 0.1 to 100 μm was selected, as well as the regular measurement mode.
The total liquid volume in the serially connected devices was derived from the conductivity of the liquid after mixing with 10 mL of 1 M KCl. By comparison with a KCl calibration curve, the internal volume was calculated to be 938 +/–13 mL.

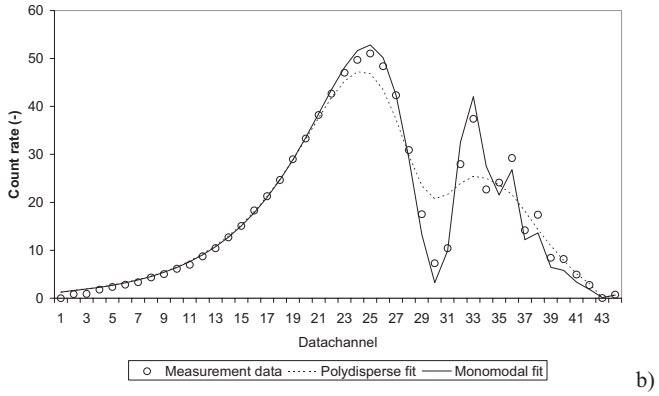
3 Results and Discussion

3.1 Robustness of Particle Size Analysis

The particle size analysis robustness of different techniques is largely dependent on *internal* parameters, such as software settings that determine the way the measured signals have to be translated into particle size distribution data. Apart from the settings of the device, *external* parameters such as experimental conditions can also clearly influence the measurement results. From the following experiments it becomes obvious that it is the combination of both internal and external parameters that determines whether the reported particle size distributions are reliable or not.
In a first experiment, PMMA latex beads were measured by SLS by either choosing the monomodal or polydisperse analysis model. An amount of a concentrated latex dispersion was added to the sample unit in order to obtain an obscuration of about 11 %, which is in the recommended range of 10 to 30 % for the Mastersizer SLS device. Figure 1a shows the markedly different results that were obtained by the two analysis methods.
For a sample of unknown composition, the polydisperse model is generally advised, which leads to the result represented by the dashed line in Figure 1a. The obtained bimodal distribution is in clear contradiction with image analysis results (represented by the photograph in the inset of Figure 1a), which reveal that the sample is characterized by a narrow monodisperse particle size distribution. Only by selecting the monomodal option in the software could a similar conclusion be derived from SLS, as indicated by the full line in Figure 1a. This experiment clearly indicates that quite different particle size distributions can be fitted to the same SLS data, in which the selection is complicated without additional knowledge of the sample (e.g. derived from alternative techniques). Since SLS is a deconvolution technique, which tries to reconstruct a measured scattering pattern by calculating the theoretical scattering pattern of an estimated particle size distribution, it is seen that the software optimization algorithm has a limited potential



a)



b)

Fig. 1: a) Differential volume weighted particle size distribution of latex beads by SLS using either a polydisperse or monomodal analysis model. The figure inset shows a microscopic image of the particles, clearly indicating the monodispersity of the sample. b) Measured and fitted angular scattering patterns for polydisperse and monomodal analysis models.

in finding the most accurate particle size distribution when selecting the general polydisperse model for samples of narrow particle size distribution. Only with the additional information supplied by the operator, by specifically indicating that the particle size distribution is monomodal is the software able to find a particle size distribution whose theoretical scattering pattern matches the measured scattering pattern very well. This is clearly seen from the residual between the scattering data and fit for the monomodal and the polydisperse model (Figure 1b), which is 1.622 % and 3.303 %, respectively. Therefore, a priori knowledge of the sample particle size distribution highly improves the ability to recover accurate particle size distributions for SLS measurements. As an alternative, the goodness of fit, expressed by a low residual value, may be used to distinguish between different possible measurement results. In any case, it is recommended to always check the fitted pattern with the measured scattering data for systematic deviations.

In a second experiment, 2 g of glass beads AQ313 added to the sample dispersion unit were measured by SLS, selecting different mathematical theories and refractive index values for the transformation of the scattering data into particle size distributions. It is seen that the choice of refractive index affects the amount of particles reported in the sub-Fraunhofer range (Figure 2), which is generally indicated as the range with particle diameters less than 20 times the wavelength of the incident light beam [5].

It was particularly observed that in some cases a submicron peak was displayed, namely when the Mie theory was selected in combination with a real refractive index of 1.48 or less, or when the Fraunhofer theory was

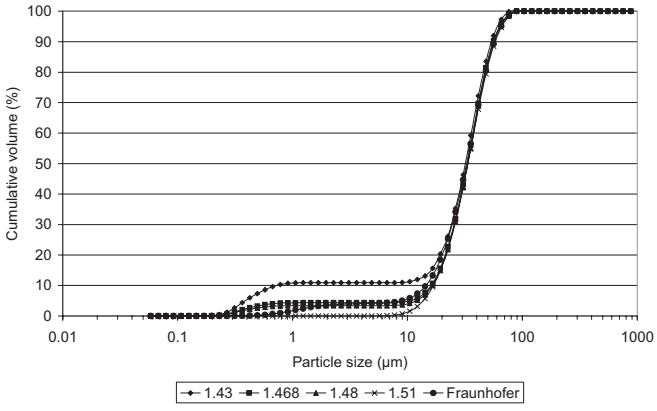


Fig. 2: Cumulative volume weighted particle size distributions obtained from SLS for a sample of 2 g of glass beads AQ313 by using different real refractive indices in the Mie model (imaginary refractive index = 0) or by using the Fraunhofer model.

selected (Figure 2). This phenomenon has already been described earlier by Hayakawa et al. [6]; they suggested selecting the particle refractive index that gave rise to the largest average particle size, i.e., with the smallest submicron peak area. Also in this experiment, it was noticed that the lowest residual between the measured scattering data and the fitted pattern corresponded to the most accurate particle size distribution.

As both SLS and TOT devices were coupled in series in this experiment, the same samples could be measured by the time of transition technique. For TOT measurements, a choice has to be made from three measurement modes: special, regular, or super regular mode. According to the CIS-100 instruction manual [7], special mode has to be selected for transparent materials, regular mode is the best choice for materials that are neither completely opaque nor completely transparent, and super regular mode is most suitable for particles that are absolutely opaque. The working mode determines what signal pulse forms are accepted as particles and how these pulses are interpreted. Figure 3a shows the mechanisms behind the different modes.

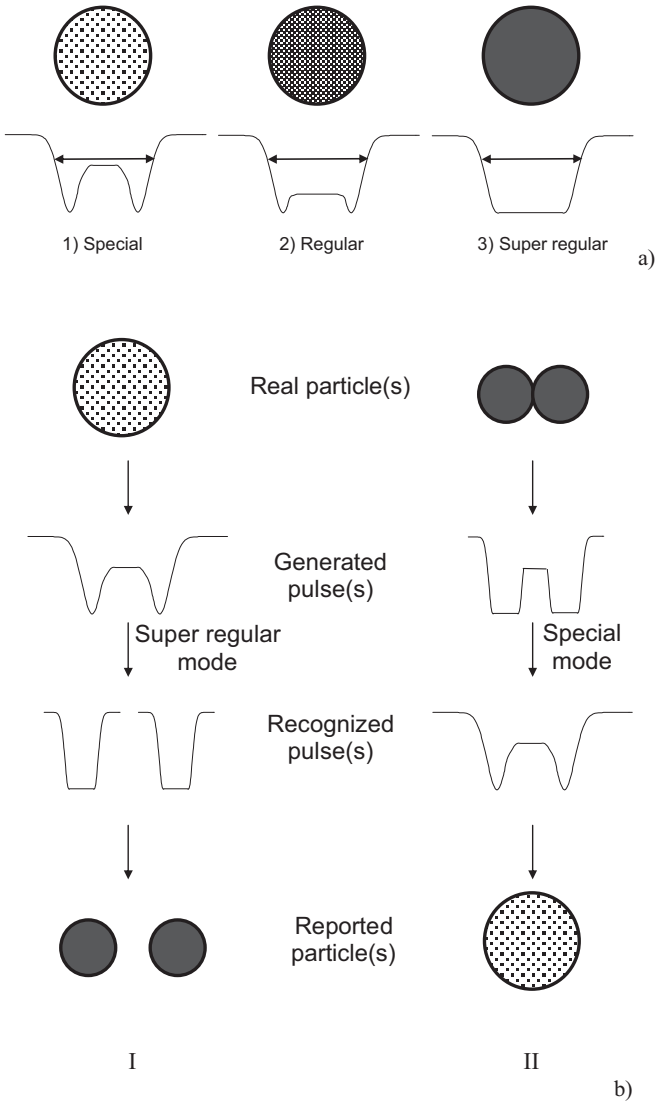


Fig. 3: a) Typical signal shapes for different particle transparencies and recommended associated measurement modes in the CIS-100 TOT-software; the arrows indicate the measured diameter of the particles. b) Schematic representation of anomalies occurring in TOT measurements when measuring transparent particles with super regular mode (I) or when measuring a concentrated sample of opaque particles with special mode (II).

When a rotating light beam passes through a transparent particle, lens effects at the edges cause a clear extinction on the light detector, whereas the body of the particle causes only partial extinction of the light beam. As such, a signal is generated, as depicted in scheme 1. With increasing opacity, the light beam passing through the body of the particle is more attenuated, leading to signal forms as drawn in schemes 2 and 3. The measurement modes special, regular and super regular are especially designed for interpreting the signal forms from schemes 1, 2 and 3, respectively.

TOT measurements, using the regular mode for signal analysis, yielded a bimodal distribution with one mode at larger particle diameters (Figure 4a).

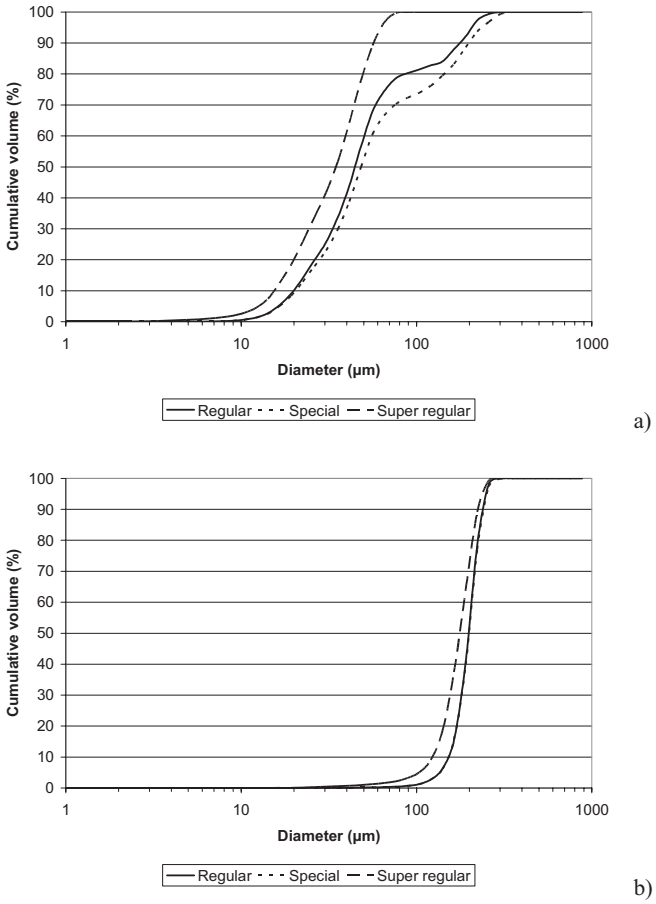


Fig. 4: Cumulative volume weighted particle size distributions generated by TOT using different measurement modes for 2 g of glass beads AQ313 (a) or AC (b).

Whereas this phenomenon became more pronounced when selecting special mode in signal analysis, selecting super regular mode eliminated it, but resulted in a particle size distribution with generally smaller particles, which was very similar to the SLS results. These artifacts, related to the choice of the measurement mode, are explained as follows. When dealing with a dispersion of (semi)transparent particles, the signal pulse caused by one particle may be interpreted as a sequence of pulses from two smaller particles when using the super regular measurement mode, as depicted in scheme I in Figure 3b. This may lead to an underestimation of the particle size. On the other hand, when dealing with small (semi)opaque particles, a signal pulse sequence as depicted in scheme II in Figure 3b may arise, especially for concentrated dispersions. When this sample is ana-

lyzed with the special measurement mode, this sequence of two unresolved signals may be erroneously attributed to one larger transparent particle rather than two closely spaced small particles. When using regular mode, one of both anomalies may occur as well, depending on the particles' transparency and concentration. For the sake of completeness, it should be mentioned that the coincidence interference is largely dependent on the particle size. Thus, a monomodal distribution corresponding to the manufacturer's specifications was obtained if 2 gram of the large glass beads AC were used using either the regular or special mode (Figure 4b). This phenomenon follows from the fact that the interparticle distance is directly proportional to the particle size at fixed volume fraction. As such, it can be calculated that for a concentration of 2 g of particles in the given sample dispersion unit, the average interparticle distance is 1733 μm for 200 μm particles, whereas it is only 173 μm for 20 μm particles. In the latter case, the risk of coincidence interference is clearly not negligible. For the large AC glass beads, it was also noticed that the use of super regular mode resulted in a measured particle size which was smaller compared to regular or special mode, and also smaller than the Mastersizer results. This might be explained by the too rigorous interpretation of the signals in super regular mode. Slightly transparent particles of a certain size will exhibit a signal as depicted in Figure 3b I, which is interpreted by the device as two signals originating from two nearby particles of a smaller size.

In a third experiment, 10 different amounts of glass beads AQ313, ranging in a logarithmic series from 0.2 up to 20 g, were added to the serially connected devices. With SLS, a small peak at several hundreds of μm was sometimes observed at the smallest particle concentrations (Table 1).

This peak may be ascribed to the fact that the experimental SLS data result from the difference between a background measurement (i.e. in the absence of particles) and a measurement in the presence of particles. As the absolute error of the difference of two experimental observations corresponds to the sum of the absolute errors of the two observations, it follows that a large relative error is obtained if the scattering due to the particles is hardly larger than the background signal. The signal that is thus generated is mainly the result of electronic noise, or of some impurities being present in the sampling unit, especially air bubbles. From about 1.5 to 4.3 gram of glass beads, similar results were obtained for the different SLS measurements. At larger particle concentrations, however, an additional mode at smaller particle diameters became increasingly important. The latter may be ascribed to multiple scattering, since this phenomenon increases the amount of light detected at larger scattering angles.

TOT measurements were made using regular mode. Table 1 shows three parameters for each measurement: SNF, SDU, and the modal diameters of the particle size distribution. The signal normalization factor (SNF) represents the light intensity reaching the cell's particle detector. Relatively low numbers indicate an over-concentration of the dispersion, whereas numbers close to 1 indicate under-concentration of the sample. The solution density uncalibrated (SDU) is a relative measurement of the concentration of particles in the mixture. It is based on the number of interactions between the laser beam and the particles, and is proportional to the sample concentration, provided that the concentration is within an acceptable range (neither too dilute, nor too concentrated) [7]. Contrary to the SLS equipment, where the optimal sample concentration is clearly defined by an obscuration level between 10 and 30 %, the optimal SNF and SDU values are sample dependent and have to be determined empirically. On the one hand, it is seen in Table 1 that for the smallest sample amounts

Table 1: Different measurement parameters for in-line particle size analysis by the SLS, TOT and DIA techniques of glass beads AQ 313 dosed to the sample delivery unit in different amounts.

Sample mass (g)	SLS obscuration (%)	SLS modes (μm)	TOT SNF (–)	TOT SDU (–)	TOT modes (μm)	DIA modes (μm)
0.200	1.6	33.3/685.7	1.00	639	38.10	41.4
0.334	3.1	37.8/705.3	1.00	1470	26.05	41.4
0.557	4.9	36.5/293.0	0.99	2467	21.54	41.4
0.928	7.8	36.8/243.0	0.92	3255	26.2/41.4	41.4
1.549	9.6	33.0	0.96	4200	26.2/222.3	41.4
2.583	14.6	31.4	0.90	6205	22.5/163.8	30.5
4.309	23.8	33.1	0.81	6893	26.2/163.8	48.3/140.6
7.188	37.5	0.31/32.4	0.57	6718	48.3/163.8	–
11.990	54.9	0.31/32.8	0.50	6583	48.3/163.8	–
20.000	77.1	0.30/32.7	0.50	6062	2.65/16.6	–

in this experiment the SNF value is 1 and the SDU value is low, suggesting under-concentration. On the other hand, from a sample mass of 0.928 g onwards, the SNF value starts decreasing rapidly and the SDU value seems to incline to a plateau value, after which it again declines with increasing sample concentration. With increasing sample concentration, it is also noticed that a bimodal distribution is generated, due to coincidence effects, suggesting over-concentration. It is deduced from these results that the optimal sample concentration for the TOT device is lower than for the SLS device and situated within a more narrow range.

Dynamic Image Analysis yielded consistent results within the range from 0.2 to 1.5 gram of glass beads, as seen from the constant mode at 41.4 μm (Table 1). At higher concentrations, measurements became more and more troublesome due to the too high concentration, which prevented particles from being well resolved in the microscopic images. From a sample mass of 7.2 g onwards, no further measurements could be performed with the DIA module.

From the results of these experiments, it follows that the results of SLS may be highly affected by the choice of the refractive index in converting the experimentally determined scattering pattern into a particle size distribution. As a simple rule, it can be stated that submicron peaks should be considered as ghost peaks if a distribution without submicron peaks provides a similar fit to the experimental data. The fact that neither TOT nor DIA require refractive index information could be considered as a clear advantage, especially for samples containing particles of different chemical composition and hence different refractive index, although it must be stressed that the transparency of the particles should be taken into account for optimal measurement results.

As far as particle concentration is considered, both TOT and DIA prefer quite low particle concentrations so that coincidence interference is prevented, and the sample should thus be diluted accordingly. When the desired dilution cannot be realized, the 'super regular' mode may be selected in order to minimize coincidence interference effects at higher particle concentrations, although this can lead to some underestimation of the particle size. As the experimental data for SLS follow from the difference of the light scattering pattern in the absence and presence of particles, it follows that this technique prefers a higher concentration, provided that multiple scattering is prevented. It should also be stated that the Mastersizer software gives a warning in case the particle concentration is too high or too low, so errors related to the concentration could be avoided to a large extent. For TOT and DIA, finding the optimal particle concentration is more empirical, and therefore more prone to operating errors.

3.2 Resolution of Bimodals

In order to check the resolution of the different techniques, samples of 2 g of mixed glass beads were brought into the sample dispersion unit, consisting of 0, 1, 2, 5, 10, 20, 50, 80, 90, 95, 98, 99, or 100 % (m/m) of AQ313 beads, the remainder being glass beads AC. The experimentally determined particle size distributions demonstrated that the smaller AQ313 particles could be detected in the presence of the larger AC particles by both SLS and DIA even if they represented only 1 % of the total mass (Figure 5b).

For the case of image analysis, the sensitivity towards a small amount of small particles follows directly from the fact that individual particles are counted. Hence, the primary information is number-weighted, and hence smaller particles are favored. For SLS, the sensitivity is mainly determined by the light scattering properties of the different particle sizes. As 2 gram of AQ313 glass beads resulted in an obscuration of 20 %, whereas 2 gram of glass beads AC gave only 2 % of obscuration, it follows that the smaller particles are more efficient scatterers and hence can be detected with a higher sensitivity. Furthermore, as can be deduced from Table 1, a sample mass of 2 g is rather low for SLS but quite high for TOT. Therefore, super regular mode was selected for the TOT measurements. However, due to the selection of the super regular mode, the TOT results always showed some shift towards smaller particles. As discussed above, this is due to the fact that the signal from a particle with some transparency may be interpreted as a signal sequence from two nearby particles with a smaller size. As a consequence, small particles were measured by TOT, even if the population consisted of 100 % large AC particles (Figure 5a).

On the other hand, at least 5 % of the larger AC particles had to be added before they could be detected by TOT in the presence of an excess of AQ313 particles (Figure 5d). For SLS and DIA at least 10 % of the larger particles had to be present to be clearly discerned (Figure 5c). For SLS, this effect is explained by the reduced scattering potential of the large particles. For DIA, the latter effect may be explained by the fact that large particles are more rapidly rejected than small particles because they are not fully situated within the field of view, or because they are out of focus. Furthermore, both DIA and TOT are counting techniques, which primarily yield number based particle size distributions. As a result of this, they show a high susceptibility to errors in reporting the presence of a small mass fraction of large particles in the particle size distribution, since these large particles only represent very low number fractions.

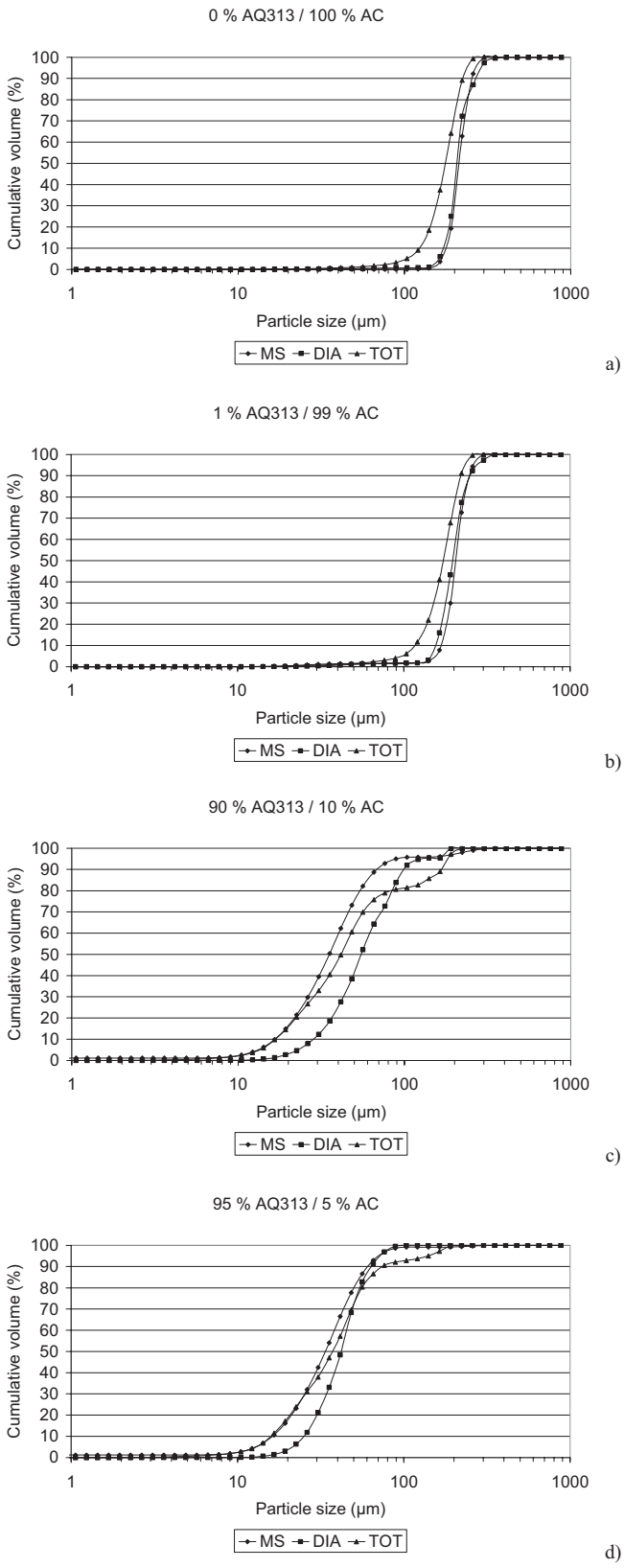


Fig. 5: Cumulative volume weighted particle size distributions by SLS, TOT and DIA coupled in series for mixtures of glass beads AQ313 and AC at a ratio of 0/100 (a), 1/99 (b), 90/10 (c) and 95/5 (d), respectively.

3.3 Lower Size Limit

In order to judge the lower particle size limit of the SLS and TOT devices, raw milk was microfluidized at different intensities, which enabled the production of samples of the same composition, only differing in particle size distribution. From a colloid-chemical point of view, milk can be considered as a dispersion of about 3.5 % (m/m) protein and 4.4 % (m/m) fat particles in an aqueous continuous phase containing lactose and salts [8]. Milk proteins consist of caseins and whey proteins; the former are organized into large aggregates of about 100 to 500 nm diameter, which are referred to as casein micelles, whereas the latter are dissolved in the continuous phase. When comparing the particle size distribution results of 10 mL of 20 times diluted raw milk (Figure 6a), it became immediately obvious that laser diffraction yielded a bimodal distribution, characterized by modal diameters of 0.28 and 3.95 µm, whereas TOT gave rise to one single mode at 4.47 µm when water was used as a dilution liquid. This experiment indicates that TOT is

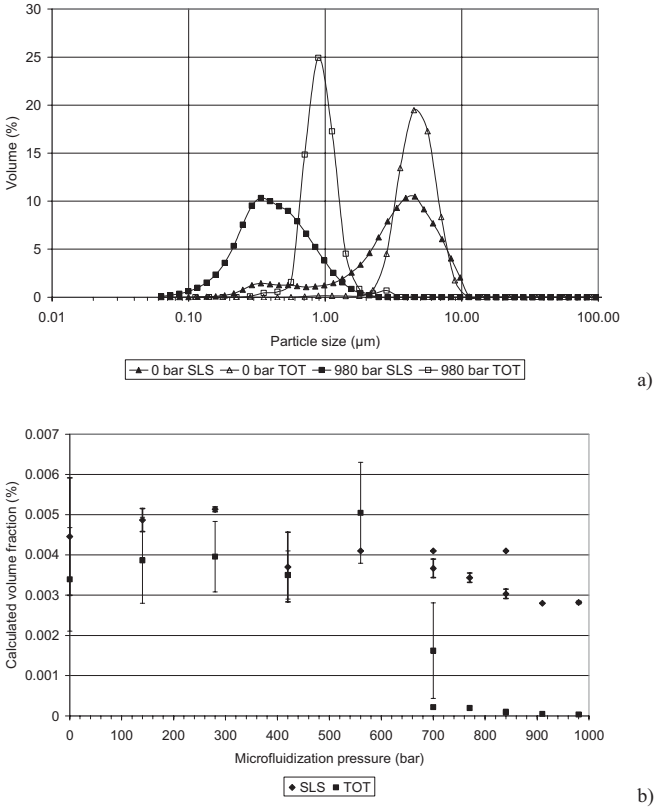


Fig. 6: Particle size analysis of 10 mL of 20 times diluted milk suspension dosed to the sample delivery unit, before and after microfluidization at different pressures. a) Differential volume weighted particle size distribution for untreated (triangles) milk and milk microfluidized at 980 bar, as obtained by SLS (filled symbols) and TOT (open symbols). b) Calculated volume fraction derived from SLS and TOT as a function of microfluidization pressure.

capable of sizing the supermicron fat particles, but is not sensitive towards the submicron casein micelles.
Subsequently, the raw milk was subjected to microfluidization in a Microfluidizer M110S during 1 minute with the aim of reducing the fat droplet particle size [9]. The homogenization pressures ranged from 0 to 980 bar, and the sample was led through an ice-water mixture to prevent excessive heating. Figure 6a shows the results of the TOT and SLS measurements of raw milk before and after homogenization at 980 bar, respectively: comparable results were obtained for raw milk before microfluidization with regard to the supermicron mode. This similarity between the TOT and SLS results remained up to a treatment at 280 bar, which did not significantly affect the size distribution. Following the most intense microfluidization, treatment at 980 bar, a significant difference was observed between the SLS and TOT results: the TOT modal diameter of 0.89 μm was about 3 times larger as compared to the laser scattering results with a modal diameter of 0.34 μm (Figure 6a). For the sake of completeness, it can be mentioned that dynamic light scattering (at a scattering angle of 90 degrees) yielded a modal diameter of 239 nm for the latter sample, which was in line with the SLS results. An even stronger indication for the fact that laser scattering is more sensitive towards submicron particles may be derived from the experimentally determined particle volume fractions (Figure 6b). At low microfluidization pressure, which mainly resulted in supermicron particle sizes, the values determined by both SLS and TOT were comparable and in line with the theoretical volume fraction of 0.0027 %. However, the experimentally determined concentration became progressively more underestimated by TOT upon increasing the microfluidization treatment, thus pointing to the fact that TOT is quite insensitive towards the smallest particles, which explains the growing discrepancy between the estimated particle size from laser scattering and TOT upon more intensive particle size reduction. From this experiment it could be derived that the lower limit of TOT was located at about 0.8 μm, whereas particles down to 0.1 μm could be measured by SLS. This example clearly showed that the experimentally determined particle concentration reported by TOT enables data quality evaluation, in which largely underestimated values indicate the presence of a pronounced fraction of hardly-detectable submicron particles. For the sake of completeness, it should be mentioned that this insensitivity towards the smallest particles could not be avoided by choosing another measurement mode in the TOT software. On the contrary, the phenomenon of a secondary peak of large particles appeared again when selecting special mode, due to the incorrect interpretation of two small particles at low distance as one larger particle, as depicted in scheme II in Figure 3b.
For DIA measurements, the lower size limit is related to the electronic resolution of the CCD camera and the magnification of the lens used. According to the manual [7], this was about 2.8 μm for lens DW. Higher magnification lenses, such as the CW lens, can reach lower size limits of 0.9 μm, but being a visible light based microscopic technique, the DIA module is not suited for submicron particles.

4 Conclusions

In this study it was demonstrated that the accuracy of experimentally determined particle size distributions is affected by several factors. Depending on the software settings and experimental conditions such as particle optical properties and concentration, markedly different results may be obtained. In order to assess the validity of the measurement results, a thorough knowledge of the possibilities and limitations of every technique is needed. Whenever possible, it is recommended to measure particle size distributions with different techniques or with different sample dilutions and software settings for a given technique. As such, anomalies occurring in the measurement results may be revealed and a higher accuracy can be obtained.

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